Na^+/H^+ exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor

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Yasutake, Masahiro, Chikao Ibuki, David J. Hearse, and Metin Avkiran. Na^+/H^+ exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor. *Am. J. Physiol.* 267 (*Heart Circ. Physiol.* 36): H2430–H2440, 1994.—Activation of sarcolemmal Na^+/H^+ exchange has been proposed as a causal factor in reperfusion arrhythmogenesis. To test this hypothesis, we determined the antiarrhythmic efficacy of two structurally distinct but equipotent Na^+/H^+ exchange inhibitors, 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) and the novel drug, 3-methylsulfonyl-4-piperidinobenzoyl guanidine (HOE-694), in isolated rat hearts (n = 12/group) subjected to independent dual coronary perfusion. After 15 min of aerobic perfusion of both beds, flow to the left coronary bed (LCB) was terminated for 10 min; this was followed by 5 min of reperfusion. Various concentrations of each drug were selectively infused into the LCB either during the 5-min period preceding ischemia plus during reperfusion or during reperfusion alone. With the former protocol, 0.01, 0.1, 1, and 10 μM EIPA reduced the incidence of reperfusion-induced ventricular fibrillation (VF) from 92% in controls to 83, 83, 50, and 0% ($P < 0.05$); the number of hearts in sinus rhythm at the end of reperfusion was increased from 17 to 42, 25, 83 ($P < 0.05$), and 100% ($P < 0.05$). HOE-694, at the same concentrations, reduced VF incidence from 92% in control to 83, 58, 50, and 8% ($P < 0.05$); 25, 67, 75 ($P < 0.05$), and 100% ($P < 0.05$) of hearts were in sinus rhythm, compared with 17% of controls, at the end of reperfusion. Even when infused during reperfusion alone, both drugs afforded significant protection against reperfusion-induced VF, which did not differ significantly from that observed when the drugs were also given before ischemia. The similar antiarrhythmic efficacy of EIPA and HOE-694 is consistent with an arrhythmogenic role for activation of Na^+/H^+ exchange during early reperfusion.

regional ischemia; dual coronary perfusion; 5-(*N*-ethyl-*N*-isopropyl)amiloride; 3-methylsulfonyl-4-piperidinobenzoyl guanidine; rat heart

RECENT STUDIES in our laboratory (2, 11) have shown that the incidence of reperfusion-induced ventricular fibrillation (VF) can be strikingly reduced by using transient acidic reperfusion to limit the rate at which extracellular H^+ is washed out. Because the sarcolemmal Na^+/H^+ exchanger is inhibited by extracellular acidosis (32), we proposed that inhibition of the exchanger was the most likely mechanism underlying the protection afforded by acidic reperfusion (2, 11). Indeed, pharmacological inhibitors of the Na^+/H^+ exchanger, such as amiloride and its analogues ethylisopropylamiloride, dimethylamiloride, and hexamethyleneamiloride, also have been shown (6, 7, 19, 25) to afford protection against reperfusion-induced arrhythmias.

Despite considerable evidence suggesting that activation of the Na^+/H^+ exchanger may be an important progenitor of reperfusion-induced arrhythmias, the interpretation of many studies is confounded by a number of factors. First, in some studies (7, 25), Na^+/H^+ exchange inhibitors were administered before the onset of ischemia, thus making it impossible to deduce whether the protective mechanisms were operative primarily during ischemia or during reperfusion. A second concern is that the chemical structures of the Na^+/H^+ exchange inhibitors used in many previous studies (6, 7, 19, 25) are based on amiloride, which is known to inhibit a number of other ion transport processes (e.g., Na^+, Ca^{2+}, and K^+ channels; Na^+/Ca^{2+} exchange) (13). Although amiloride analogues, in which the 5-amino nitrogen atom bears one or two substituents, can exhibit increased potency as inhibitors of the Na^+/H^+ exchanger (13), the possibility cannot be excluded that nonselective actions might contribute to their protective effects. A final concern is that, although acidic reperfusion (2, 11) is likely to inhibit the Na^+/H^+ exchanger (32), it is likely also to exert other actions (e.g., inhibition of Ca^{2+} and K^+ channels, Na^+/Ca^{2+} exchange, and Ca^{2+} release from the sarcoplasmic reticulum) that may contribute to an antiarrhythmic effect (2).

The present study was designed with the primary objective of obtaining stronger evidence for a specific involvement of the Na^+/H^+ exchanger in the induction of reperfusion arrhythmias by a mechanism that is operative primarily during the reperfusion phase. To achieve this, we used as pharmacological tools an amiloride analogue that is a potent inhibitor of the Na^+/H^+ exchanger, 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA), and compared its effects with those of a recently developed inhibitor, 3-methylsulfonyl-4-piperidinobenzoyl guanidine (HOE-694) (24), which has a chemical structure distinct from that of amiloride and its analogues (Fig. 1). To obtain a detailed assessment of the potency and efficacy of these drugs and to determine their primary phase of action, we used four concentrations of each agent and two treatment protocols, one in which the drug was given before ischemia and during reperfusion and the other in which it was given during reperfusion alone. Another novel aspect of the present study was our decision to administer selectively the drugs only to the zone that was subjected to ischemia and reperfusion; this was made possible by our recent development (1) of a unique cannula that allows the independent perfusion of left and right coronary beds in small mammalian hearts. In further studies using Langendorff-perfused hearts, we have also investigated the effects of EIPA and HOE-694 on contractile function and electrocardiographic parameters under aerobic conditions.

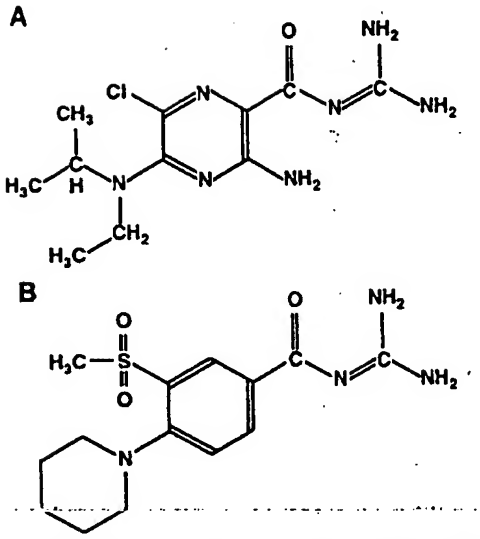

Fig. 1. Chemical structures of 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA; *A*) and 3-methylsulfonyl-4-piperidinobenzoyl guanidine (HOE-694; *B*).

METHODS

This investigation was performed in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, published by Her Majesty's Stationery Office, London, UK.

Arrhythmia Study

Dual coronary perfusion of isolated rat hearts. Isolated hearts from male Wistar rats (Bantin and Kingman, North Humberside, UK) were subjected to independent perfusion of left and right coronary arteries as described in detail by Avkiran and Curtis (1). Each coronary bed was initially perfused at a constant perfusion pressure equivalent to 75 mmHg with oxygenated perfusion solution from a temperature-regulated reservoir (37°C). The perfusion solution was of the following composition (in mM): 118.5 NaCl, 25.0 $NaHCO_3$, 3.2 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 1.4 $CaCl_2$, and 11.0 glucose. The solution was filtered (pore size, 5 μm) before use and bubbled continuously with 95% O_2-5% CO_2 (pH 7.4 at 37°C). Flow to each coronary bed was monitored using in-line flow detectors (Transonic T206 Animal Research Flowmeter with 1N probes, Transonic Systems, Ithaca, NY) with a linear detection range of 0.05–30 ml/min. After 15 min of perfusion of both coronary beds, basal flow rate in the left coronary bed was recorded, and perfusion of this bed was switched from constant pressure to constant flow at the basal flow rate (supplied by a Gilson Minipuls 3 roller pump). This enabled the infusion of drug solutions or vehicle into the left coronary bed, at a known percentage of the total flow supplied to that bed (see *Drug administration and study protocol*). The right coronary bed was perfused at constant pressure throughout the experiment. The heart was housed in a temperature-regulated chamber at 37°C throughout the experiment, and the right atrium was continuously superfused with oxygenated perfusion solution (37°C) at 8 ml/min to maintain sinus rate (1).

Drug administration and study protocol. HOE-694 has limited solubility in physiological buffer solutions. Therefore, both drugs were dissolved in deionized water at concentrations of 0.143, 1.43, 14.3, and 143 μM. When required, these solutions were infused selectively into the perfusion line supplying the left coronary bed at 7% of the total flow rate delivered to that bed; this resulted in final perfusate drug concentrations of 0.01, 0.1, 1, and 10 μM. Control hearts received vehicle (deionized water) at the same infusion rate.

Each concentration of EIPA or HOE-694 was infused into the left coronary bed either for 5 min immediately before ischemia plus throughout reperfusion or throughout reperfusion alone. All hearts (n = 12/group) were subjected to 10 min of regional ischemia (this was achieved by terminating flow to the left coronary bed) followed by 5 min of reperfusion. Experiments were carried out in a prospectively randomized manner.

Measured variables. ARRHYTHMIAS. Arrhythmias were diagnosed from a unipolar electrogram (ECG) that was obtained through a silver electrode inserted into the free wall of the left ventricle and a reference electrode connected to the aorta. The ECG was continuously monitored on a digital storage oscilloscope (model 1421, Gould Electronic, Ilford, UK) and was recorded on an ink-jet recorder (model 2200S, Gould). Chart speed was set at 50 mm/s a few seconds before reperfusion so as to obtain a permanent high-speed record of the changes in the ECG during early reperfusion. The ECG was retrospectively analyzed, in a blinded manner, for the incidence, time to onset, and duration of ventricular tachycardia (VT) and VF. All analyses were carried out in accordance with the Lambeth Conventions (30). VT was defined as four or more consecutive premature beats of ventricular origin and VF as a signal in which individual QRS deflections could no longer be distinguished from one another and for which rate could not be determined.

VT CYCLE LENGTH. VT cycle length was determined 10 s after the onset of reperfusion, since at this time point a large proportion of the hearts (n = 10–12) were in VT in most groups, enabling intergroup statistical comparison. The only exception was the group that received 10 μM EIPA before ischemia plus during reperfusion; none of the hearts in this group exhibited VT (see RESULTS). VT cycle length was calculated from the number of QRS deflections over a 2-s interval, using the high-speed ECG trace (2).

CORONARY FLOW AND HEART RATE. Throughout the experimental protocol, coronary flow was monitored using the in-line flow detectors. Heart rate was determined at selected intervals from the ECG trace.

SIZE OF ISCHEMIC ZONE. At the end of each experiment, the left coronary bed was perfused for 30 s with a solution containing 0.02% disulphine blue dye at a perfusion pressure of 75 mmHg. The heart was then removed from the perfusion apparatus, the atria and mediastinal tissue were removed, and the dye-stained tissue (representing ventricular myocardium subjected to ischemia and reperfusion) was carefully dissected out. The stained and unstained tissues were lightly blotted and weighed. The size of the ischemic zone, expressed as a percentage of total ventricular weight, was calculated from the equation: (weight of stained tissue/total ventricular weight) × 100. The absolute weights obtained also enabled the calculation of flows in left and right coronary beds on the basis of tissue weights supplied by each bed ($ml \cdot min^{-1} \cdot g^{-1}$).

Exclusion criteria. These criteria, selected to minimize variations in heart rate and size of ischemic zone (due to atypical coronary anatomy) among the hearts, were as previously described (1, 2, 10, 11). Hearts were also excluded if there was cross-flow between right and left coronary ostia, due to a mismatch between aorta size and cannula diameter (1, 2, 10, 11). In addition, hearts that exhibited ventricular arrhythmias during the final 3 s of ischemia before reperfusion were not included in the analysis of reperfusion-induced arrhyth-

mias, because in those hearts it would have been impossible to differentiate arrhythmias induced by reperfusion from those induced by ischemia. Of 230 hearts entered into the arrhythmia study, 5 were excluded on the basis of heart rate, 1 on the basis of size of ischemic zone, 4 on account of cross flow, and 4 on account of arrhythmias during the final 3 s of ischemia. The overall exclusion rate was 6%.

Contractile Function Study

Langendorff perfusion of isolated rat hearts. Hearts were excised from male Wistar rats as described in an earlier study (1) and were subjected to Langendorff perfusion through a conventional single-lumen cannula at a constant perfusion pressure equivalent to 75 mmHg. The left atrium was excised, and an ultrathin balloon, specially constructed to match the dimensions of the ventricular cavity, was inserted into the left ventricle. The intraventricular balloon was inflated to give a left ventricular end-diastolic pressure (LVEDP) of 6–8 mmHg, and the balloon volume was kept constant thereafter. The perfusion solution was identical to that used for dual coronary perfusion. After 15 min of perfusion at constant pressure, the basal total coronary flow rate was noted, and perfusion was switched to a constant-flow system at the basal flow rate. Again, 7% of the total flow supplied was from a sidearm through which drug solutions or vehicle could be administered.

Drug administration and study protocol. For the first 10 min of constant-flow perfusion, hearts (n = 6/group) received vehicle (deionized water) through the sidearm. Subsequently, drug solutions were administered, in a cumulative manner, to give final perfusate concentrations of 0.01, 0.1, 1, and 10 μM, and perfusion with each drug concentration was maintained for 10 min. Hearts in the control group continued to receive vehicle (deionized water), at the same infusion rate, throughout the protocol.

Measured variables. CONTRACTILE FUNCTION. LVEDP and left ventricular developed pressure (LVDP), obtained from a pressure transducer attached to the intraventricular balloon through a fluid-filled catheter, were noted at 2-min intervals during perfusion with each drug concentration.

CORONARY VASCULAR RESISTANCE (CVR) AND HEART RATE. CVR was determined at 2-min intervals during perfusion with each drug concentration, from the total coronary flow (which was constant) and the perfusion pressure (which was monitored through a sidearm of the aortic cannula). Heart rate was determined at the same intervals from the ECG trace (see below).

ECG PARAMETERS. At the end of the 10-min perfusion period with each drug concentration, chart speed was increased to 250 mm/s to obtain a permanent, high-speed ECG recording. These recordings were utilized to measure the interval from the beginning of the P wave to the beginning of the ventricular complex (P-R interval) and the width of ventricular complex. As a separate T wave is not seen in the rat ECG, the width of the ventricular complex was measured at 90% repolarization (with the maximum positive deflection of the ventricular complex defined as the point of 0% repolarization) and defined as $QRST_{90}$, as previously described (21).

EXCLUSION CRITERIA. These prospectively defined criteria demanded that hearts were excluded if basal heart rate was <280 or >420 beats/min or if basal total coronary flow rate was <9 ml/min. Of 18 hearts entered into the contractile function study, none were excluded.

Statistical Analysis

Statistical analyses were based on the guidelines described by Wallenstein et al. (31). Gaussian-distributed variables were expressed as means ± SE and were subjected to one-way analysis of variance. If a difference among mean values was established, comparison with controls was performed using Dunnett's test. Temporal changes in heart rate, developed pressure, and coronary vascular resistance were analyzed using analysis of variance for repeated measurements. Binomially distributed variables, such as the incidence of VT or VF, were compared using the chi-squared test for a $2 \times n$ table. If a significant difference was revealed, each drug-treated group was then compared with the control group using the Fisher exact test, with the Bonferroni correction for multiple comparisons. A value of $P < 0.05$ was considered significant.

RESULTS

Arrhythmia Study

Reperfusion-induced arrhythmias. Consistent with our earlier studies (2, 11), reperfusion after 10 min of regional ischemia frequently resulted in the rapid (within a few beats) induction of VT. Reperfusion-induced VT was generally polymorphic in nature, and episodes of VT were usually uninterrupted until either spontaneous reversion to normal sinus rhythm or degeneration into VF.

DRUG ADMINISTRATION BEFORE ISCHEMIA PLUS DURING REPERFUSION. Figure 2 shows the overall incidence of reperfusion-induced VF in control hearts and hearts that received EIPA or HOE-694 before ischemia plus during reperfusion. Both drugs suppressed the incidence of VF in a concentration-dependent manner, the reduction reaching a level of statistical significance at a

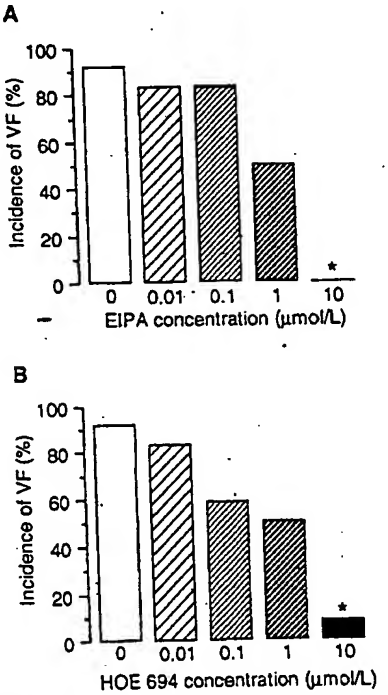

Fig. 2. Effects of EIPA (*A*) and HOE-694 (*B*) when given before ischemia plus during reperfusion on overall incidence of reperfusion-induced ventricular fibrillation (VF). * $P < 0.05$ vs. 0 μM (control).

concentration of 10 μM in both cases. Figure 3 illustrates the incidence of VF in each of the study groups during 0.5-min intervals throughout the 5-min reperfusion period. The time-to-onset of reperfusion-induced VF was not altered by either drug, with the induction of VF occurring during the first 0.5 min of reperfusion in the majority (67–100%) of hearts that exhibited VF in the various study groups.

Although the overall incidence of VF was reduced only by the highest concentration (10 μM) of each drug, in the groups that received 1 μM EIPA or 0.1 μM HOE-694 there were significant reductions in the incidence of VF during some of the later time intervals during reperfusion (Fig. 3). This probably reflected the ability of the drugs to enhance spontaneous defibrillation at these lower concentrations. The concentration-response characteristics of EIPA and HOE-694 with respect to their antifibrillatory actions appeared to be similar (Figs. 2 and 3), with half-maximal protection obtained within the 0.1–1 μM concentration range in both cases. The maximal protection afforded was also similar for both drugs, indicating comparable efficacy. The incidence of reperfusion-induced VT was 100% in all control and drug-treated groups, except in the group that received 10 μM EIPA in which reperfusion-induced VT or VF was not detected. In both control groups, only 17% of

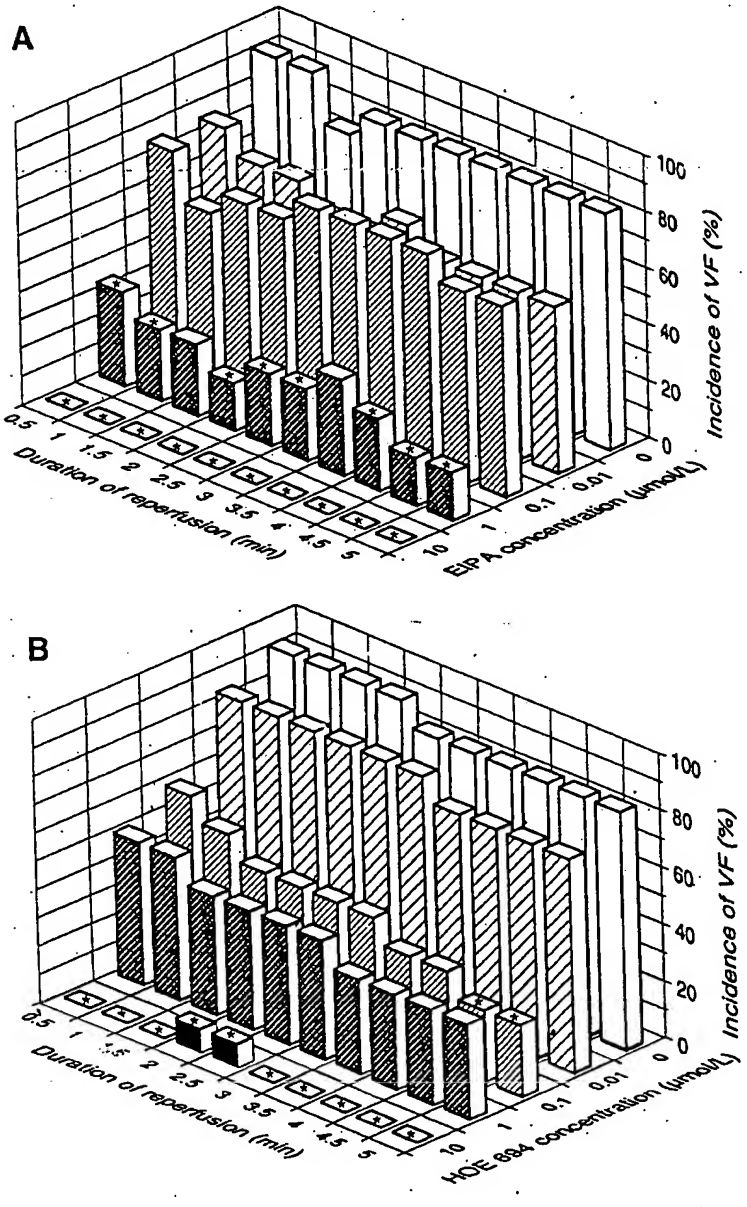

Fig. 3. Effects of EIPA (*A*) and HOE-694 (*B*) when given before ischemia plus throughout reperfusion on time course of reperfusion-induced VF. Incidence of VF was noted during 0.5-min intervals throughout the 5-min reperfusion period. *$P < 0.05$ vs. 0 μM (control) during corresponding interval.

hearts were in normal sinus rhythm at the end of the reperfusion period. In the groups that received 0.01, 0.1, 1, and 10 μM EIPA before ischemia and during reperfusion, 42, 25, 83 ($P < 0.05$), and 100% ($P < 0.05$) of hearts, respectively, were in sinus rhythm at the end of reperfusion. The corresponding values in the groups that received HOE-694 were 25, 67, 75 ($P < 0.05$), and 100% ($P < 0.05$), respectively.

DRUG ADMINISTRATION DURING REPERFUSION ALONE. Figure 4 shows the time course of reperfusion-induced VF in the groups that received EIPA and HOE-694 during reperfusion alone and in the contemporary control groups. Both drugs again suppressed the incidence of VF and enhanced spontaneous reversion to normal sinus rhythm in a concentration-dependent manner. The reductions in the overall incidence of VF, relative to the control values of 92%, reached statistical significance at the concentration of 10 μM for both HOE-694 (25%) and EIPA (33%). HOE-694 at 0.01 μM tended to enhance spontaneous defibrillation, as indicated by the significant reductions in the incidence of VF during some of the later time intervals during reperfusion (Fig. 4); however, the overall incidence of VF was not significantly reduced by this concentration. The concentration-response profiles for the drugs (Fig. 4) suggest that HOE 694 may exhibit greater potency than EIPA when given

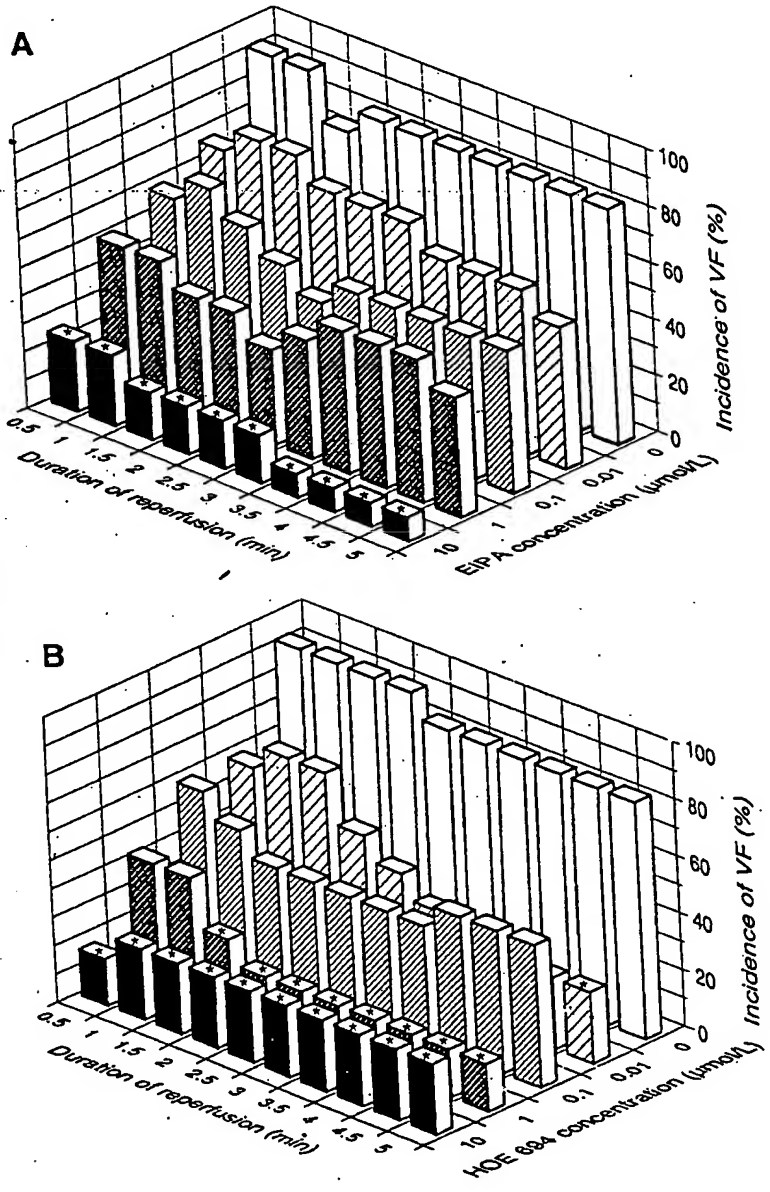

Fig. 4. Effects of EIPA (*A*) and HOE-694 (*B*) when given during reperfusion alone on time course of reperfusion-induced VF. Incidence of VF was noted during 0.5-min intervals throughout 5-min reperfusion period. *$P < 0.05$ vs. 0 μM (control) during corresponding interval.

during reperfusion alone; however, the efficacy of the drugs appeared comparable, with similar protection afforded by the highest concentration (10 μM) of each agent. The incidence of VT was 100% in all groups, regardless of the concentration or identity of the drug. In both control groups, only 17% of hearts were in normal sinus rhythm at the end of reperfusion. In the groups that received 0.01, 0.1, 1, and 10 μM of EIPA during reperfusion, this incidence was increased to 50, 50, 50, and 83% ($P < 0.05$), respectively. The corresponding values in the groups that received identical concentrations of HOE-694 during reperfusion were 75 ($P < 0.05$), 50, 92 ($P < 0.05$), and 92% ($P < 0.05$), respectively.

VT cycle length. As shown in Fig. 5, when administered before ischemia plus during reperfusion, both drugs produced a concentration-dependent prolongation of VT cycle length, when measured 10 s after the initiation of reperfusion. In the group that received 10 μM of EIPA before ischemia plus during reperfusion, VT cycle length could not be measured because none of the hearts exhibited reperfusion-induced VT. VT cycle length during early reperfusion was not significantly different from control when EIPA and HOE-694 were administered during reperfusion alone, regardless of drug concentration.

Coronary flow, heart rate, and ischemic zone size. As shown in Table 1, there was no significant difference between control and drug-treated groups in basal left and right coronary flow rates, when measured at the end of the initial 15-min period of perfusion at constant pressure. Thereafter, left coronary flow rate was held constant at the basal value; therefore, there were no significant intergroup differences in left coronary flow rate for the rest of the experimental protocol. Flow rate in the right coronary bed (which was perfused at constant pressure throughout) did not change significantly during the period of zero-flow ischemia in the left coronary bed. Right coronary flow rate increased during reperfusion commensurate with the severity of reperfusion arrhythmias; this was probably due to reduced extravascular compression, as previously described (2, 11).

Table 1. *Basal coronary flow, heart rate, and ischemic zone size in the 18 groups included in the arrhythmia study*

Concentration, μM	Treatment Protocol	Coronary Flow Rate, ml·min⁻¹·g⁻¹ LCB	Coronary Flow Rate, ml·min⁻¹·g⁻¹ RCB	Heart Rate, beats/min	Ischemic Zone Size, %
		EIPA			
0 (control)		13.6±0.5	14.0±0.7	350±11	60±2
0.01	pre-I+R	14.7±1.1	16.8±1.0	330±5	57±2
0.1	pre-I+R	13.3±0.7	17.1±1.3	345±9	60±3
1	pre-I+R	13.1±0.8	16.2±1.1	352±10	60±2
10	pre-I+R	14.3±0.9	16.4±0.7	356±12	56±3
0.01	R	13.9±0.7	16.4±1.3	333±10	56±3
0.1	R	13.0±0.9	15.6±1.1	368±15	55±2
1	R	13.7±0.6	16.6±1.2	346±14	61±3
10	R	13.7±0.8	16.8±0.6	340±11	60±3
		HOE-694			
0 (control)		11.6±0.3	14.6±0.6	322±7	61±2
0.01	pre-I+R	11.0±0.4	15.9±0.8	334±9	62±3
0.1	pre-I+R	11.7±0.5	16.2±0.6	338±6	63±2
1	pre-I+R	10.3±0.5	14.3±0.7	323±9	63±2
10	pre-I+R	13.1±0.4	15.2±0.7	352±8	56±2
0.01	R	11.1±0.4	13.9±0.7	321±7	64±2
0.1	R	12.1±0.7	15.6±1.1	339±8	61±2
1	R	10.9±0.6	14.5±0.9	336±7	59±2
10	R	13.1±0.6	15.3±0.8	338±7	56±2

Values are means ± SE; $n = 12$ rat hearts/group. LCB and RCB, left and right coronary beds, respectively; pre-I, before ischemia; R, during reperfusion; EIPA, 5-(*N*-ethyl-*N*-isopropyl)amiloride; HOE-694, 3-methylsulfonyl-4-piperidinobenzoyl guanidine.

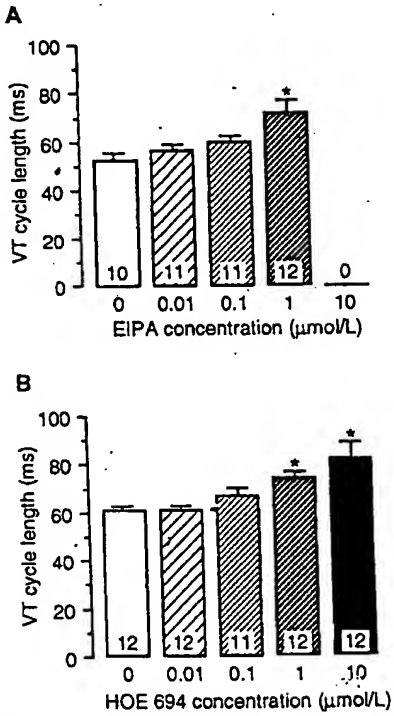

Fig. 5. Effects of EIPA (*A*) and HOE-694 (*B*) when given before ischemia plus during reperfusion on cycle length of ventricular tachycardia (VT) determined 10 s after onset of reperfusion (see METHODS). Nos. in columns indicate group size (0 hearts exhibited VT in group that received 10 μM EIPA). * $P < 0.05$ vs. 0 μM (control).

Basal heart rate also did not differ significantly between control and drug-treated groups (Table 1). The infusion of EIPA or HOE-694 into the left coronary bed for 5 min immediately before the onset of ischemia had no effect on heart rate, regardless of drug concentration. Heart rate did not change significantly in any of the study groups during the period of regional ischemia and could not be measured during early reperfusion due to the rapid onset of ventricular arrhythmias in the majority of hearts. There was no difference between control and drug-treated groups in the size of the ischemic zone (Table 1).

Contractile Function Study

Contractile function, CVR, and heart rate. Throughout the study, coronary flows in the control, EIPA, and

HOE-694 groups were kept constant at their basal values of 16.2 ± 0.8, 16.4 ± 0.6, and 16.7 ± 0.5 $ml \cdot min^{-1} \cdot g^{-1}$, respectively [not significant (NS)]. Basal values (measured after 10 min of constant pressure perfusion) for LVEDP were 7.0 ± 0.1, 7.0 ± 0.2, and 7.0 ± 0.2 mmHg in the control, EIPA, and HOE-694 groups, respectively (NS). The corresponding values for LVDP were 106 ± 3, 105 ± 3, and 119 ± 3 mmHg, respectively (NS). LVEDP did not change significantly during the remainder of the experimental protocol in any of the study groups. The temporal changes in LVDP are shown in Fig. 6. There was a small decline in LVDP with time in the control group (Fig. 6*A*). There was also a small decline in LVDP with increasing concentration of HOE-694 (Fig. 6*C*); however, the similarity with the control group suggests that this was a time-dependent rather than a drug-induced effect. In contrast, EIPA resulted in a steeper decline in LVDP during infusion of the highest concentration (Fig. 6*B*) such that by the end of the experimental protocol LVDP in this group was only 48% of its basal value.

Basal values for CVR were 6.8 ± 0.2, 6.5 ± 0.2, and 6.3 ± 0.1 $mmHg \cdot ml^{-1} \cdot min^{-1}$ in the control, EIPA, and HOE-694 groups, respectively (NS). There was a small increase in CVR during the first 20 min of constant flow perfusion in the control group; thereafter, CVR remained stable (Fig. 6*A*). The temporal changes in CVR in the HOE-694 group resembled those in the control group (Fig. 6*C*). In the EIPA group, however, CVR increased further with increasing concentration of drug such that by the end of drug infusion CVR was 170% of its basal value (Fig. 6*B*).

Basal values for heart rate were also similar in the control, EIPA, and HOE-694 groups at 323 ± 17, 330 ± 12, and 315 ± 9 beats/min, respectively (NS). Heart rate did not change thereafter in the control (Fig. 6*A*) and HOE-694 (Fig. 6*C*) groups but started to decline during the infusion of 1 μM EIPA and reached 75% of its basal value following the infusion of 10 μM EIPA (Fig. 6*B*).

ECG parameters. In control, EIPA, and HOE-694 groups, basal values for P-R interval were 39 ± 3, 41 ± 3, and 37 ± 2 ms, respectively (NS), and the corresponding values for $QRST_{90}$ were 57 ± 3, 58 ± 3, and 62 ± 3 ms, respectively (NS). P-R interval and $QRST_{90}$ did not change significantly throughout the protocol in control and HOE-694 groups. During the infusion of 0.01, 0.1, and 1 μM EIPA, these parameters also did not change significantly; however, after exposure to 10 μM EIPA, P-R interval was prolonged to 73 ± 5 ms ($P < 0.05$) and $QRST_{90}$ to 126 ± 12 ms ($P < 0.05$). One of the six hearts that received EIPA developed second-degree (2 to 1) atrioventricular (AV) block.

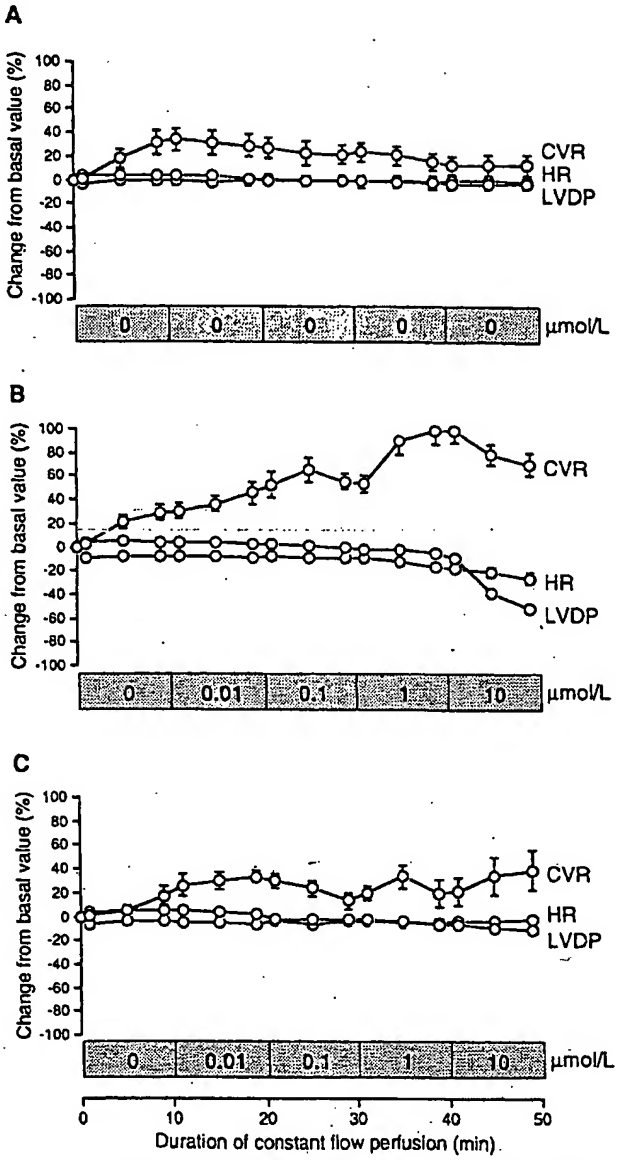

Fig. 6. Temporal changes in left ventricular developed pressure (LVDP), coronary vascular resistance (CVR), and heart rate (HR) during infusion of vehicle (control; *A*), EIPA (*B*), or HOE-694 (*C*). Stippled sections at bottom of *A-C* indicate period of constant-flow perfusion. Nos. in stippled sections indicate drug concentration (μM), with 0 denoting vehicle infusion.

DISCUSSION

The present study has demonstrated that, in isolated rat hearts, the selective intracoronary administration of EIPA or HOE-694 into the zone subjected to ischemia and reperfusion affords substantial protection against reperfusion-induced VF. EIPA and HOE-694 exhibit both antifibrillatory and defibrillatory properties, with similar potency and efficacy. These effects are observed even when the agents are administered during reperfusion alone, thus indicating the involvement of a mechanism that is operative primarily during the reperfusion phase.

Possible Mechanism(s) of Action of EIPA and HOE-694

Na^+/H^+ exchange inhibition. Activation of Na^+/H^+ exchange during early reperfusion has been proposed to result in the intracellular accumulation of Na^+ and consequently an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]$) via inhibition or reversal of the sarcolem-

mal Na^+/Ca^{2+} exchanger (15). There is indeed evidence that in the rat heart increased cellular Ca^{2+} uptake occurs during reperfusion, and this can be attenuated by pharmacological inhibition of the Na^+/H^+ exchanger (28). Such a disturbance of Ca^{2+} homeostasis has been proposed as a progenitor of reperfusion-induced arrhythmias, probably through a mechanism that involves the oscillatory release of Ca^{2+} from the sarcoplasmic reticulum and the subsequent induction of delayed afterdepolarizations (17). Indeed, in the light of their activation mapping studies in the feline heart, Pogwizd and Corr (20) have suggested that the induction of VF during reperfusion may be mediated by a nonreentrant mechanism that involves Ca^{2+} overload-mediated afterdepolarizations and triggered activity. In support of such a mechanism, the recent studies of Kihara and Morgan (12) in aequorin-loaded ferret hearts have provided evidence that reperfusion after 20 min of ischemia may result in an increase in intracellular $[Ca^{2+}]$, which is associated with the transition to VF. Thandroyen et al. (29), using isolated spontaneously beating ventricular myocytes, have also suggested that increased intracellular $[Ca^{2+}]$ may be a causal factor in the degeneration of VT into VF. In addition to its proposed role in the induction of VF, there is evidence to suggest that increased intracellular $[Ca^{2+}]$ may be important also for the maintenance of VF (12, 29). In the light of the above, the inhibition of Na^+ influx through Na^+/H^+ exchange and subsequent Ca^{2+} overload through Na^+/Ca^{2+} exchange could have mediated both the antifibrillatory and the defibrillatory effects of EIPA and HOE-694 observed in the present study.

PROTECTION BY NA^+/H^+ EXCHANGE INHIBITION DURING REPERFUSION. It has been suggested (24, 25) that the preischemic administration of Na^+/H^+ exchange inhibitors could attenuate tissue injury by inhibiting Na^+ influx during the early minutes of ischemia [before the development of significant extracellular acidosis, which itself would be expected to inhibit the Na^+/H^+ exchanger (32)]. Therefore, it could be argued that under conditions in which Na^+/H^+ exchange inhibitors are administered before ischemia, inhibition of reperfusion-induced arrhythmias may occur, at least in part, as a consequence of reduced ischemic injury. In the present study, EIPA and HOE-694 suppressed reperfusion-induced VF not only when they were administered before ischemia and during reperfusion but also when they were given during reperfusion alone. This observation provides strong evidence to suggest that the protective effect was mediated primarily by drug action during the reperfusion phase and supports the hypothesis that the activity of the Na^+/H^+ exchanger during early reperfusion is an important determinant of the severity of reperfusion-induced arrhythmias. Indeed, our studies with transient acidic reperfusion (2, 11) suggest that, in this model, inhibition of Na^+/H^+ exchange for the first 2 min of reperfusion may be sufficient to provide sustained protection against VF.

STRUCTURE-ACTIVITY AND SPECIFICITY CONSIDERATIONS. In several cell types, EIPA and HOE-694 have been shown to exhibit similar potency as inhibitors of Na^+/H^+ exchange (23, 24). The similar concentration-response profiles of the two agents observed in the present study are consistent with the hypothesis that Na^+/H^+ exchange inhibition is the primary and common mechanism underlying their ability to protect against reperfusion-induced VF. Although HOE-694 is not an amiloride analogue, it contains an acyl guanidine moiety in common with both amiloride and EIPA. Structure-activity studies with amiloride analogues in fibroblasts (14) and cardiac myocytes (15) have revealed that this moiety is important for Na^+/H^+ exchange inhibitory activity, with derivatives that contain a substituted guanidino group (e.g., benzamil and dichlorobenzamil) exhibiting much reduced potency. The acyl guanidine moiety is also present in other agents that interfere with Na^+ transport, such as tetrodotoxin, and it has been suggested that this moiety may facilitate drug interaction with a site involved in Na^+ transport (15).

Recent cloning and sequencing studies (18, 33) have shown that there are at least four isoforms of the plasma membrane Na^+/H^+ exchanger (NHE-1, NHE-2, NHE-3, and NHE-4), which may exhibit differing responsiveness to inhibition by amiloride and its analogues (4). Only the ubiquitous NHE-1 isoform (18) and, to a lesser extent, the NHE-2 (33) and NHE-3 (18) isoforms appear to be expressed in the intact adult rat heart. Within the context of the present study, it should be noted that HOE-694 has been shown recently (4) to exhibit significantly greater selectivity than amiloride and its 5-amino-substituted derivatives for NHE-1, the predominant Na^+/H^+ exchanger isoform expressed in the rat heart.

VT cycle length. In our earlier acidic reperfusion study (2), we reported a pH-dependent prolongation of VT cycle length during early reperfusion and proposed that slowing of VT may be a mechanism through which acidic reperfusion exerts its antifibrillatory action (by enhancing spontaneous reversion of VT to sinus rhythm and inhibiting its deterioration to VF). In the present study, the administration of EIPA and HOE-694 before ischemia plus during reperfusion resulted in a concentration-dependent prolongation of VT cycle length, when measured 10 s after the onset of reperfusion. This prolongation of VT cycle length correlated well with the antifibrillatory effects of both drugs, suggesting a causal role. However, the administration of EIPA or HOE-694 during reperfusion alone did not affect VT cycle length but nonetheless had a protective effect comparable to that achieved when the drugs were also given before ischemia. This would suggest that prolongation of VT cycle length during early reperfusion is unlikely to be the primary mechanism by which these Na^+/H^+ exchange inhibitors exert their antifibrillatory effects.

Nonselective effects. In the present study, exposure of the hearts to cumulatively increasing concentrations of EIPA resulted in negative inotropic and chronotropic effects and increased vascular resistance; this is consistent with previous observations with EIPA in the guinea pig heart (19). During infusion of the 10 μM concentration, EIPA also prolonged the P-R interval and $QRST_{90}$, indicative of abnormalities in AV nodal conduction (in one heart AV block was observed) and ventricular

depolarization and/or repolarization. It is well established that amiloride (and to a lesser extent its analogues such as EIPA) inhibit a number of other ion transport processes (e.g., Na^+, Ca^{2+}, and K^+ channels, Na^+/Ca^{2+} exchange) (13) and can have multiple electrophysiological effects (8). In this regard, previous in vitro studies have shown that amiloride can slow AV nodal conduction without affecting intraventricular conduction (34), have a negative chronotropic effect on the sinoatrial node (22), and prolong the action potential duration in Purkinje fibers (16). Interestingly, the pronounced effect on repolarization in Purkinje fibers was manifest only after prolonged exposure to amiloride, suggesting that this effect may be mediated by the intracellular accumulation of the drug (16). In a similar manner, the cardiodepressant and electrocardiographic effects of EIPA observed in the present study were evident only during infusion of the 10 μM concentration, following prolonged (30 min) exposure to the lower concentrations of the drug. Therefore, nonselective actions (perhaps mediated by intracellular drug accumulation) may have contributed to the protective effects of high concentrations of EIPA observed in the present study, when the drug was administered before ischemia (thus allowing extended exposure to the drug trapped in the ischemic bed). Indeed, with this treatment protocol, 10 μM EIPA totally abolished reperfusion-induced arrhythmias (including VT), a property not shared by HOE-694. However, nonselective effects are less likely to have played a major role in the protective effects of EIPA against reperfusion-induced arrhythmias when the drug was infused during reperfusion alone, where the duration of exposure to the drug was limited.

Although detailed electrophysiological data on HOE-694 are not currently available, some observations from the present study suggest that relative to EIPA, HOE-694 may exhibit fewer nonselective effects. Thus, unlike EIPA, prolonged exposure to increasing concentrations of HOE-694 (up to 10 μM) in the absence of ischemia and reperfusion did not affect heart rate, contractile function, or ECG parameters. This would be the expected profile of a selective Na^+/H^+ exchange inhibitor which, in bicarbonate-buffered medium under control conditions, should not affect intracellular pH or other ion transport mechanisms.

Relevance to the Mechanism of Reperfusion-Induced Arrhythmias

The results of the present study indicate that the activation of Na^+/H^+ exchange during reperfusion is an important arrhythmogenic factor; they do not, however, preclude a significant role for other factors [e.g., washout of extracellular K^+ (5), generation of oxygen free radicals (3)] in reperfusion-induced arrhythmogenesis. Indeed, whereas EIPA and HOE-694 significantly inhibited reperfusion-induced VF in a concentration-dependent manner, the incidence of reperfusion-induced VT remained at 100% in most groups, thus supporting the argument that multiple factors are involved. The only exception was the group that received 10 μM EIPA before ischemia plus during reperfusion, in which arrhythmias were not detected; however, the nonselective effects of EIPA (discussed in *Nonselective effects*) may have contributed to this phenomenon. It is also possible that some factors associated with reperfusion, such as free radical-induced oxidant stress, may act in a synergistic manner with activation of Na^+/H^+ exchange to disrupt Ca^{2+} homeostasis (9).

Myocardial ischemia results in the neuronal release and extracellular accumulation of catecholamines within the ischemic zone, through locally mediated mechanisms (26). Previous studies (27) have shown that pharmacological inhibition of the Na^+/H^+ exchanger markedly suppresses this ischemia-induced catecholamine release. In the light of the established arrhythmogenic potential of adrenergic stimulation, it is probable that inhibition of ischemia-induced catecholamine release may have contributed to the protective effects of EIPA and HOE-694 in the present study, particularly when the drugs were given before ischemia.

Limitations of the Study

The present study utilized the known pharmacological characteristics of two structurally distinct drugs to investigate the role of the Na^+/H^+ exchanger in reperfusion arrhythmogenesis. Because EIPA and HOE-694 have in common the ability to inhibit the Na^+/H^+ exchanger in an equipotent manner, it is reasonable to ascribe their similar protective effects against reperfusion-induced arrhythmias in the present study to inhibition of the exchanger. However, on the basis of this study, it cannot be deduced whether the protection was mediated by reduced intracellular accumulation of Na^+ and/or Ca^{2+} during reperfusion.

One limitation of the present study may be the use of an erythrocyte-free perfusion medium, which results in coronary flows in excess of those encountered in vivo; such high flows may result in faster washout during reperfusion of components (such as H^+) that have accumulated during the preceding period of ischemia, which in turn may lead to an overestimation of the role of washout phenomena in reperfusion arrhythmogenesis. However, it is worth noting that in the present model even a 90% reduction in the rate of reflow is unable to suppress reperfusion-induced VF (10).

It may be argued also that the use of the rat heart, which has unusual electrophysiological characteristics (such as a short action potential duration and a high heart rate), may limit the applicability of the present findings to other species. Although this is acknowledged, it must be pointed out that the model used in the present study offers many advantages in the study of the pathophysiological determinants of arrhythmogenesis (1), such as the consistent generation of regional ischemia and arrhythmias, the ability to use multiple drug concentrations and groups of adequate size (due to the low cost of the preparation), and the ability to infuse drugs selectively into the ischemic-reperfused zone.

Concluding Comments

The present study has shown that in isolated rat hearts subjected to regional ischemia, the selective infusion into the ischemic-reperfused zone of EIPA or HOE-694, two structurally distinct but equipotent inhibitors of the Na^+/H^+ exchanger, significantly suppresses the induction of VF during reperfusion and promotes spontaneous reversion to normal sinus rhythm. The drugs exhibit similar potency and efficacy and are effective even when given during reperfusion alone. The protective effects are observed in the absence of intergroup differences in the size of the ischemic zone, heart rate before and during ischemia, the rate of reflow, and oxygen tension of the perfusate. These findings are consistent with an arrhythmogenic role for activation of Na^+/H^+ exchange during the early moments of reperfusion.

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